

Abstract

Bc. Hana Hřebíková

Identification of membrane localized native peptides of intracellular bacteria *Francisella tularensis*

Diploma thesis

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Specialist in Laboratory Methods

Background: The main goal of this diploma thesis was develop new methods suitable for assume of concentration of peptides in biological sample and identification of membrane-associated proteins of bacteria *Francisella tularensis* (*F. tularensis*).

Methods: Experimental development was tested on standard proteins like alfa kasein and BSA. These proteins had known concentration and they were treated by trypsin. The concentration of reached peptides were measured by modified and classic method of micro BCA (bicinchoninic acid protein assay). Method was applied on real bacterial sample of *F. tularensis* species strain FSC200. By cultivation of whole bacteria bacterial peptides were prepared. The cultivation was provided in different time intervals in solution, which contained trypsin. This method named shaving was described in literature.

Results: The concentration of peptides was measured by classic micro BCA method. Peptides were separated by HPLC and identified by mass spectrometry. Identification of two bacterial peptides from protein named 17 kDa major membrane protein (ZP_0227498) confirmed right choice of used method.

Conclusions: The next step followed this diploma thesis will focused on preparation of membrane-reached fraction by „shaving“ method and identification of another membrane proteins of *F. tularensis*.